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Rapid Communication

Eukaryotic initiation factor 5A-1 (eIF5A-1) as a diagnostic marker for aberrant proliferation in intraepithelial neoplasia of the vulva

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Abstract

Objective. The mature eukaryotic translation initiation factor 5A contains the unusual amino acid hypusine, formed post-translationally from a specific lysine residue and essential for proliferation of eukaryotic cells. We hypothesized that the major eIF5A isoform, eIF5A-1, is an in situ biomarker for proliferation. NIH-353, a polyclonal immunoreagent specific for hypusine-containing eIF5A-1, was used to test this proposal in biopsies of vulvar high-grade intraepithelial neoplasia (VIN), characterized by the presence of proliferating cells throughout the thickness of the epithelium.

Methods. Formalin-fixed and paraffin-embedded archival samples with an independently established diagnosis of VIN 3 were stained immunohistochemically after antigen retrieval, employing NIH-353 and, for comparison, the standard Ki-67 antibody.

Results. NIH-353 labeled neoplastic keratinocytes throughout the thickness of the epithelium in all VIN 3 samples. Malignant cells in a case of focally invasive squamous cell carcinoma also stained strongly for mature, hypusine-containing eIF5A-1. Epithelium adjacent to these lesions, though still of apparently normal morphology, was immunoreactive throughout its full thickness. At inflammatory foci of lesional sites, solitary reactive lymphocytes were positive, as were individual proliferating cells within dermal appendages. The submucosal stroma lacked reactive cells.

Conclusion. NIH-353 identifies mature eIF5A-1 as an in situ biomarker for proliferation. Like Ki-67, this immunoreagent promises broad applicability in histopathological diagnosis and may be helpful in outcome prediction. In contrast to Ki-67, NIH-353 visualizes a molecular target for antineoplastic therapy, and thus may guide the development and clinical testing of drugs that, like the fungicide ciclopirox, inhibit hypusine formation and cell proliferation.

Keywords: EIF5A-1; Intraepithelial neoplasia; Vulva

Introduction

Genetic information is necessary for protein assembly. However, it is not always sufficient to secure the proper folding and the physiological function of a protein. Often, post-translational modifications are required, which transform the covalent structure of genetically encoded, peptide-bound amino acids into novel residues [1]. For instance, the sequences of interstitial collagens prominently display 4-hydroxyproline, sometimes in every third position, although this residue, which determines the helical structure of collagens, is not specified by the genetic information encoding the collagens. Similarly, post-translationally formed amino acid residues such as the iodotyrosine moiety of thyroglobulin and the 4-carboxyglutamate moiety of coagulation factors II, VII, IX, and X determine the biological function of these proteins. In each of these examples, an inactive precursor becomes a biologically active protein only after undergoing post-translational modifications.

The eukaryotic translation initiation factor 5A (eIF5A) also contains an amino acid not specified by genetic infor-

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mation. This unusual residue is hypusine, the product of two sequential post-translational modifications. Hypusine originates from a specific lysine moiety by butylamine transfer utilizing spermidine, and by subsequent hydroxylation utilizing atmospheric oxygen [2]. Extensive evidence indicates that among eukaryotes, hypusine serves an essential function in the control of cell proliferation. The residues surrounding the hypusine moiety remain entirely conserved throughout eukaryotic evolution, from archaea over fungi and plants to insects and vertebrates [2]. In yeast, the single mutation of the lysine precursor to hypusine abolishes its capacity to proliferate [3]. In cultures of normal or malignant human cells, reversible suppression of hypusine formation correlates with reversible arrest of the cell cycle in late G1 [4]. Mature, hypusine-containing eIF5A was reported to promote the association of a sub-set of proliferation-related mRNAs with polysomes [5] and also the nucleocytoplasmic transport of certain mRNAs [6]. Putative motifs in such hypusine-dependent mRNAs (*hymns*) have been proposed to exist in their untranslated regions, for example, in the mRNAs that encode cyclin D1 ([7]; manuscript in preparation) or cyclooxygenase-2 [8], proteins known to function in exit from G1 and in carcinogenesis, respectively.

Two human isoforms of eIF5A exist, eIF5A-1 and eIF5A-2. Both isoforms harbor the hypusine modification [9]. The first isoform, eIF5A-1, is ubiquitously expressed and abundant in proliferating cells. In contrast, the second isoform, eIF5A-2, is expressed only in specific tissues or in certain cancer cell lines. Recently, we observed that NIH-353, a rabbit polyclonal antibody raised against human mature eIF5A-1, displays a high specificity toward fully modified, hypusine-containing eIF5A-1 [10], and negligible cross reactivity with eIF5A-2 [9]. For these reasons, we considered NIH-353 to be a suitable tool for detecting the presence of mature eIF5A-1 in proliferating cells of human tissues.

In a preliminary study [10], we noted that the NIH-353 immunoreagent stained the cells in the basal and parabasal regenerative layers of normal cervical epithelium in a pattern similar to the one obtained immunohistochemically for the nuclear protein Ki67, a widely employed marker for proliferating cells of all tissues and expressed only during cell cycle progression [11]. We therefore chose to examine the potential utility of NIH-353 in established cases of intraepithelial neoplasia of the vulva (VIN), the precursor to vulvar cancer [12]. VIN is not only a model for the proliferative abnormalities caused by infection of oral and anogenital mucosa with oncogenic types of human papillomavirus (HPV) [13], but is also representative of intraepithelial neoplasia in general, the near-obligate antecedent to cancer in most epithelial tissues [14].

Materials and methods

Polyclonal antiserum against the native hypusine-containing eIF5A-1, purified from human erythrocytes [15], was generated in a rabbit and termed 'NIH-353'. The specificity of NIH-353 was established by Western blot (NuPage™ Bis-Tris Electrophoresis System; Invitrogen Life Technologies, Carlsbad, CA) using side-by-side comparison of its reactivity with two biosynthetic forms of eIF5A-1, that is, the lysine-containing precursor eIF5A-1 (Lys) and the mature hypusine-containing form eIF5A-1. A monoclonal mouse antibody against the carboxyterminal segment of eIF5A-1 that lacks the hypusine-containing loop and comprises residues 58 to 154 was used to assess hypusine-independent immunoreactivity of eIF5A-1 (BD-6119; BD Biosciences, San Jose, CA). The two antibodies were compared by Western blot using the two forms of eIF5A, eIF5A-1(Lys), and eIF5A-1(Hpu). Signal was developed with a chemiluminescence reagent (SuperSignal West Pico Substrate; Pierce Biotechnology, Rockford, IL) on film (BioMax MR™; Kodak, Rochester, NY). The polyclonal, affinity-purified rabbit antiserum against the human Ki67 protein that was employed for immunohistochemistry detects a 20-residue epitope of the protein Ki67 (Ki-67; Dako, Glostrup, Denmark) and displays reactivity similar to the mouse monoclonal MIB-1 variant. The UMDNJ Institutional Review Board for Human Subjects Protection approved the anonymous use of archival, formalin-fixed, paraffin-embedded vulvar tissue, containing either normal skin or lesions that had received an independently established diagnosis of VIN 3. A total of nine tissue blocks were studied in this exploratory investigation, three normal and six lesional samples. The plates shown in Fig. 2 summarize the representative findings. In these tissues, signal generation with NIH-353 and with Ki-67 required the application of a cycled microwave irradiation protocol for antigen retrieval, using a commercially available buffer system (Citra™; BioGenex, San Ramon, CA). Immunostaining of proliferating cells in formalin-fixed paraffin-embedded human tissues was optimal at a 1/80 dilution of NIH-353. In identically prepared sections of the same tissue blocks that were to be evaluated as negative controls, the first antibody (NIH-353 or Ki-67, respectively) was omitted. For signal generation, the streptavidin-biotin/horseradish peroxidase complex technique was used, with diaminobenzidine as chromogen and hematoxylin as counterstain. Endogenous peroxidase was blocked in all tissue sections as described [16]. All tissue sections were counterstained with hematoxylin.

Results

The selectivity of NIH-353 for the mature hypusine-containing form of eIF5A-1 was shown by Western analysis (Fig. 1). This reagent recognized the mature protein at 0.01 and 0.1 μg (lanes 1 and 2), while the lysine-containing precursor was hardly detectable even at 1 μg (lane 4). NIH-353 therefore must react with either the hypusine residue itself, or with a neoepitope that emerges as the result of the

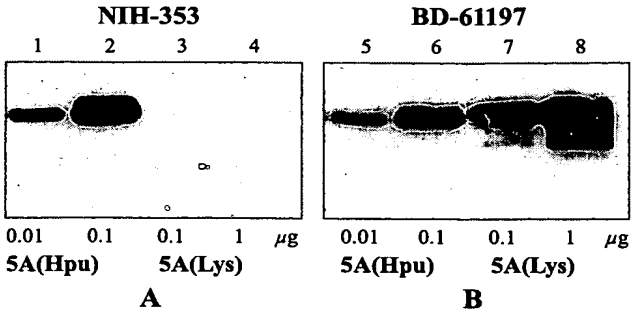

Fig. 1. Reactivity of NIH-353 (A) and of BD-61197 (B), with hypusine-containing eIF5A-1 [5A(Hpu)] and with its lysine precursor [5A(Lys)]. Purified proteins were electrophoresed in parallel lanes (1–4 and 5–8) on a 12% Nu-PAGE gel and transferred to a nitrocellulose membrane. The membrane was cut into two equal parts and immunostained with NIH-353 (A) or BD-61197 (B).

post-translational synthesis of hypusine. In contrast, BD-61197 failed to distinguish between hypusine-containing eIF5A-1 and its lysine-containing precursor. Raised against only the C-terminal part of eIF5A-1 (residues 58–154), which lacks the hypusine modification site, BD-61197 recognized both the lysine precursor as well as the hypusine form of eIF5A-1 with similar signal intensity (Fig. 1B). The lack of reactivity of the lysine-containing precursor [5A(Lys)] on panel A (lanes 3 and 4) with NIH-353 was apparently not due to the absence of the antigen protein on the membrane, since the two lanes (3 and 4) gave a strong signal upon reprobing with BD-61197 (data not shown).

The NIH-353 reagent, reactive with the biologically active form of eIF5A-1, exclusively labeled the keratinocytes in the basal and parabasal layers of normal vulvar skin (Fig. 2A). The Ki-67 reagent, known to visualize the Ki67 protein as it occurs in the nucleus during S phase and in the perichromosomal region during mitosis [17], likewise labeled keratinocytes exclusively in these epidermal strata (Fig. 2C). These strata are the only intraepithelial sites of proliferative activity, supplying the cells that are physiologically required for regeneration and maintenance of normal squamous epithelium.

In intraepithelial neoplasia of the vulva (VIN 3), Ki-67 decorated the nuclei of keratinocytes in all layers of the epidermis, revealing the presence of the Ki67 protein and thus, on-going proliferation. Positive cells were often arranged in clusters. In the subepithelial matrix, the few reactive cells located preferentially to inflammatory foci below the basement membrane at lesional sites and were identified as reactive lymphocytes (Fig. 2D). NIH-353 generated an analogous pattern of signal distribution. The aggregate of keratinocytes throughout the entire thickness of the epithelium was positive (Fig. 2B), as were individual

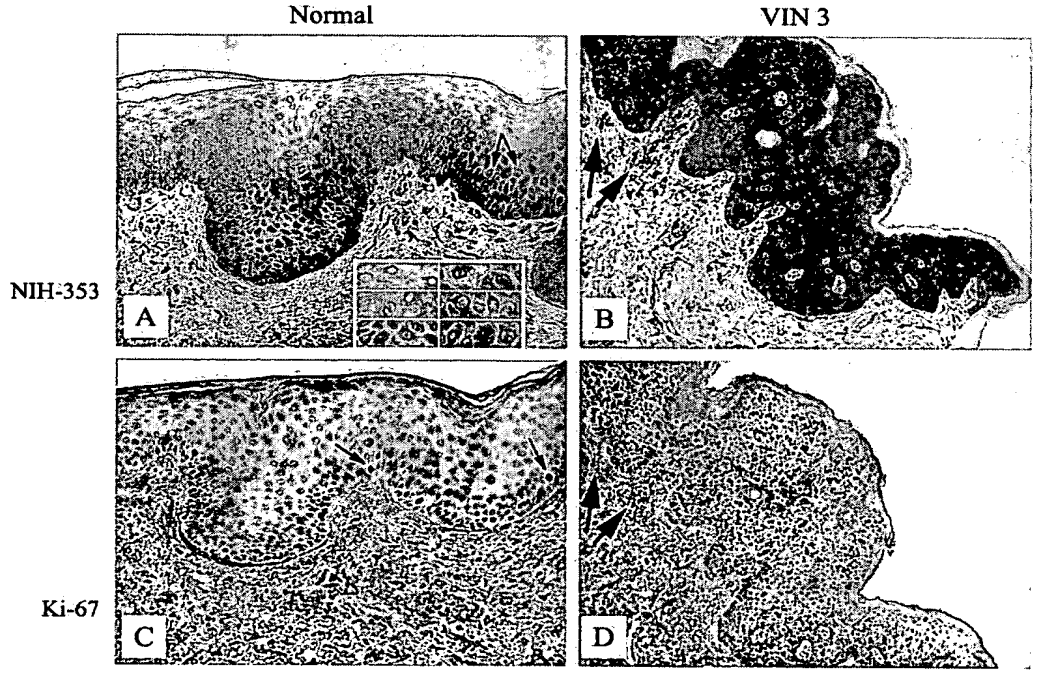

Fig. 2. Immunohistochemical staining with NIH-353 and Ki-67, respectively, of healthy vulvar skin (Normal) and of a Grade 3 vulvar intraepithelial neoplasia (VIN 3). Identical sections of normal vulvar skin (A, C) are shown at high power, and identical sections of a typical VIN 3 lesion (B, D) at low power. NIH-353 was used in the upper panels (A, B) and Ki-67 in the lower panels (C, D). Small arrows highlight individual cells that exemplify the extranuclear staining by NIH-353 (A; also high power inset into A) and the intranuclear staining by Ki-67 (C). Large arrows highlight isolated aggregates of reactive lymphocytes immediately below the basement membrane that are similarly detected by NIH-353 (B) and by Ki-67 (D).

cells within sweat glands or hair follicles, and reactive lymphocytes, known to synthesize hypusine-containing eIF5A-1 [18] (Fig. 2, arrows in B/D). In contrast to Ki-67, whose intranuclear localization generated a punctate signal (Fig. 2, arrows in C), the NIH-353 stain did not highlight the nuclei and instead, detected the presence of hypusine-containing eIF5A-1 in the cytoplasm, often in a preferentially perinuclear localization (Fig. 2, arrows in A). The often distinctly visible yet uniform exclusion of the NIH-353 signal from nuclei generated unstained nuclear lacunae. Only in mitotic cells, in which the nuclear envelope had been dissolved, did the NIH-353 signal distribute homogeneously, surrounding and directly abutting their chromosomal plates. In contrast to the punctate and 'periodic' intraepithelial decorations by Ki-67, the labeling by NIH-353 painted the intraepithelial areas containing proliferating cells in a more homogeneous, 'continual' manner, and occurred even in areas non-reactive with Ki-67 (compare A vs. C and B vs. D in Fig. 2). A further distinction was noted in juxtalesional epithelium not yet displaying cytological abnormalities. Whereas the intraepithelial labeling with Ki-67 reverted sharply from full-thickness to basal-layer only, NIH-353 sustained the uniform labeling of keratinocytes in the middle and upper epithelial layers, just as in morphologically manifest lesions (data not shown). This is consistent with the 'field change' around VIN lesions: adjacent vulvar epithelium of normal cytological appearance nevertheless expresses VIN-typical epitopes that are absent from healthy epithelium [19]. One of the vulvar biopsies also contained a focally invasive squamous cell carcinoma, which displayed the same distribution of mature eIF5A-1 as described for intraepithelial neoplasia (data not shown).

The submucosal stroma lacked positive cells, generating in VIN 3 cases a sharp demarcation of the Ki-67 signal and of the NIH-353 signal at the basement membrane (Fig. 2). Both immunoreagents failed to label the superficial hyperkeratotic stratum of the epidermis.

Discussion

In intraepithelial neoplasia of the vulva, the immunohistochemical detection of the Ki67 protein and of hypusine-containing eIF5A-1, using the Ki-67 and the NIH-353 immunoreagents, respectively, generated a staining pattern that clearly identifies the abnormally proliferating keratinocytes throughout all layers of the epidermis. Ki-67 staining of VIN lesions has significantly improved the accuracy of their grading [20] and has been shown to be diagnostically useful in separating vulvar HPV-related lesions from their non-HPV-related histological mimics [21]. After progression of VIN 3 lesions to vulvar squamous cell carcinoma, Ki-67 staining generates distinct distributions of lesional labels that have prognostic significance [22] and directly relate to overall survival [23]. For vulvar malignancies, the identification of novel biomarkers of diagnostic and predictive value is being actively pursued [24]. Based on our immunohistochemical findings with NIH-353, we propose that hypusine-containing eIF5A-1 should be ranked among candidate biomarkers for the in situ detection of proliferative abnormalities, for example, those triggered by oral or anogenital infections of keratinocytes with oncogenic strains of HPV.

The staining patterns observed in tissues and cells with the Ki-67 and the NIH-353 reagents are as expected. Both reagents stain actively proliferating cells, and the intracellular distribution of the signal is consistent with the subcellular prominence of their respective target proteins Ki67 and hypusine-containing eIF5A-1, that is, nuclear versus cytoplasmic, respectively. For replication of their DNA, cycling cells require both Ki67 protein and mature eIF5A-1. Rendering either protein non-functional, for example, by antisense oligonucleotides in case of Ki67 protein [25] or by inhibitors of hypusine formation in case of eIF5A-1 [2,4], disrupts cell cycle progression, decreasing the number of cells that enter into S phase, and concurrently increasing the number of cells caught in G1. Both macromolecules also show a distinct relationship with ribosomes. The Ki67 protein is an organizer of nucleoli, the site of ribosomal biogenesis [26]. Mature eIF5A-1 has been proposed to be a mediator of translational control, directing nucleocytoplasmic transport and thus the polysomal utilization of specific mRNAs [5,6]. We consider NIH-353 as the prototype of improved immunoreagents with wide applicability in histopathological diagnosis, just as the original Ki-67 gave rise to optimized antibodies, for example, of the MIB or TEC series [17].

In contrast to Ki-67 and other immunoreagents detecting proliferating cells in sections of human tissue, NIH-353 visualizes an epitope that is the molecular target of chemical compounds already in agricultural and clinical use for their remarkable antiproliferative activity. The biochemical pathway of hypusine formation has been established in great detail, facilitating the identification of several classes of inhibitors for the two enzymes involved, deoxyhypusine synthase and deoxyhypusine hydroxylase (e.g., Refs. [2,4,27,28]). Among the most potent inhibitors of deoxyhypusine hydroxylase known at present, is the therapeutically used fungicide ciclopirox, which at low-micromolar concentrations abolishes the formation of hypusine in human eIF5A-1 and causes arrest of cell proliferation in late G1 [5,28,29,30]. We recently proposed that topical application of ciclopirox, commercial preparations of which contain millimolar quantities, offers the prospect of a novel treatment principle for the medical control of intraepithelial neoplasia in general [29,30], widely considered a major challenge in cancer prevention [14]. The antineoplastic mode of action of ciclopirox is distinct from that of other compounds explored for the topical treatment of VIN, such as imiquimod [31]. The demonstration that hypusine-containing eIF5A-1 is highly

expressed in the abnormally proliferating keratinocytes of VIN 3 lesions (Fig. 2B) provides a compelling rationale for the development and clinical testing of topical drugs that, like ciclopirox, inhibit hypusine formation and consequently, the proliferation of neoplastic keratinocytes within the intraepithelial compartment.

NIH-353 not only identifies the aberrant cell proliferation of vulvar, but also of cervical intraepithelial neoplasia as well as of invasive cervical and endometrial cancers [32]. The NIH-353 reagent and its monoclonal analogs, therefore, are likely to visualize the presence of hypusine-containing eIF5A-1 in other human neoplasias.

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References

[1] Mann M, Jensen ON. Proteomic analysis of post-translational modifications. Nat Biotechnol 2003;21:255–61.
[2] Park MH, Lee YB, Joe YA. Hypusine is essential for eukaryotic cell proliferation. Biol Signals 1997;6:115–23.
[3] Schnier J, Schwelberger HG, Smit-McBride Z, Kang HA, Hershey JW. Translation initiation factor 5A and its hypusine modification are essential for cell viability in the yeast *Saccharomyces cerevisiae*. Mol Cell Biol 1991;11:3105–14.
[4] Hanauske-Abel HM, Park MH, Hanauske A-R, Popowicz AM, Lalande M, Folk JE. Inhibition of the G1-S transition of the cell cycle by inhibitors of deoxyhypusine hydroxylation. Biochim Biophys Acta 1994;1221:115–24.
[5] Hanauske-Abel HM, Slowinska B, Zagulska S, Wilson RC, Staiano-Coico L, Hanauske AR, et al. Detection of a sub-set of polysomal mRNAs associated with modulation of hypusine formation at the G1-S boundary. Proposal of a role for eIF-5A in onset of DNA replication. FEBS Lett 1995;12(366):92–8.
[6] Rosorius O, Reichart B, Kratzer F, Heger P, Dabauvalle MC, Hauber J. Nuclear pore localization and nucleocytoplasmic transport of eIF-5A: evidence for direct interaction with the export receptor CRM1. J Cell Sci 1999;112:2369–80.
[7] Hanauske-Abel HM, Hanauske AR, Slowinska B, Popowicz AM. The cell cycle-controlling protein eIF-5A: identification of functional regions relevant for interaction with its mRNA partners. FASEB J 2002;16:A549.
[8] Parker MT, Gerner EW. Polyamine-mediated post-transcriptional regulation of COX-2. Biochimie 2002;84:815–9.
[9] Clement PM, Henderson CA, Jenkins ZA, Smit-McBride Z, Wolff EC, Hershey JW, et al. Identification and characterization of eukaryotic initiation factor 5A-2. Eur J Biochem 2003;270:4254–63.
[10] Hanauske-Abel HM, Heller D, Wolff EC, Hameed M, Clement PMJ, Park MH, et al. Eukaryotic translation initiation factor 5A, an emerging target for cytostatic compounds, localizes to proliferative regions in human tissue. Cancer Epidemiol Biomarkers Prev 2002;11:1145s.
[11] Schmidt MH, Broll R, Bruch HP, Duchrow M. Proliferation marker pKi-67 affects the cell cycle in a self-regulated manner. J Cell Biochem 2002;87:334–41.
[12] Madelaine MM, Daling JR, Tamimi HK. Vulva and vagina. In: Franco EL, Rohan TE, editors. Cancer precursors. Epidemiology, Detection, and Prevention. New York: Springer; 2002. p. 321–32.
[13] Syrjänen KJ, Syrjänen SM. Papillomavirus infections in human pathology. Chichester: Wiley; 1999.
[14] O'Shaughnessy JA, Kelloff GJ, Gordon GB, Dannenberg AJ, Hong WK, Fabian CJ, et al. Treatment and prevention of intraepithelial neoplasia; an important target for accelerated new agent development. Recommendations of the American Association for Cancer Research Task Force on the Treatment and Prevention of Intraepithelial Neoplasia. Clin Cancer Res 2002;8:314–46.
[15] Park MH, Liu TY, Neece SH, Swiggard WJ. Eukaryotic initiation factor 4D. Purification from human red blood cells and the sequence of amino acids around its single hypusine residue. J Biol Chem 1986;261:14515–9.
[16] van Hoeven KH, Kovatich AJ, Oliver RE, Nobel M, Dunton CJ. Protocol for immunocytochemical detection of SIL in cervical smears using MIB-1 antibody to Ki-67. Mod Pathol 1996;9:407–12.
[17] Brown DC, Gatter KC. Ki67 protein: the immaculate deception? Histopathology 2002;40:2–11.
[18] Cooper HL, Park MH, Folk JE. Posttranslational formation of hypusine in a single major protein occurs generally in growing cells and is associated with activation of lymphocyte growth. Cell 1982;29:791–7.
[19] Naik R, Cross P, de Barros Lopes A, Robson P, Monaghan J. Lectins in the vulva: I. Normal vulvar epithelium and epithelium adjacent to vulvar intraepithelial neoplasia and squamous cell carcinoma. Int J Gynecol Pathol 1998;17:154–61.
[20] van Beurden M, de Craen AJ, de Vet HC, Blaauwgeers JL, Drillenburg P, Gallee MP, et al. The contribution of MIB 1 in the accurate grading of vulvar intraepithelial neoplasia. J Clin Pathol 1999;52:820–4.
[21] Logani S, Lu D, Quint WG, Ellenson LH, Pirog EC. Low-grade vulvar and vaginal intraepithelial neoplasia: correlation of histologic features with human papillomavirus DNA detection and MIB-1 immunostaining. Mod Pathol 2003;16:735–41.
[22] Hendricks JB, Wilkinson EJ, Kubilis P, Drew P, Blaydes SM, Munakata S. Ki-67 expression in vulvar carcinoma. Int J Gynecol Pathol 1994;13:205–10.
[23] Hantschmann P, Lampe B, Beysiegel S, Kurzl R. Tumor proliferation in squamous cell carcinoma of the vulva. Int J Gynecol Pathol 2000;19:361–8.
[24] Salmaso R, Zen T, Zannol M, Perin D, Marchiori S, Marchetti M. Prognostic value of protein p53 and Ki-67 in invasive vulvar squamous cell carcinoma. Eur J Gynaecol Oncol 2000;21:479–83.
[25] Duchrow M, Schmidt MH, Zingler M, Anemuller S, Bruch HP, Broll R. Suppression of cell division by pKi-67 antisense-RNA and recombinant protein. Cell Physiol Biochem 2001;11:331–8.
[26] Schmidt MH, Broll R, Bruch HP, Bogler O, Duchrow M. The proliferation marker pKi-67 organizes the nucleolus during the cell cycle depending on Ran and cyclin B. J Pathol 2003;199:18–27.
[27] Lee YB, Park MH, Folk JE. Diamine and triamine analogs and derivatives as inhibitors of deoxyhypusine synthase: synthesis and biological activity. J Med Chem 1995;38:3053–61.
[28] Hoffman BD, Hanauske-Abel HM, Flint A, Lalande M. A new class of reversible cell cycle inhibitors. Cytometry 1991;12:26–32.
[29] Clement PMJ, Hanauske-Abel HM, Wolff EC, Kleinman HK, Park MH. The antifungal drug ciclopirox inhibits deoxyhypusine and pro-

line hydroxylation, endothelial cell growth, and angiogenesis in vitro. Int J Cancer 2002;100:491–8.

[30] Clement PMJ, Hanauske-Abel HM, Heller DS, Wolff EC, Hardegen N, Park MH, et al. A candidate drug for chemoprevention of cervical cancer: antiproliferative effects of the fungicide ciclopirox in the cervical cancer line SiHa. Abstr. # 1157. 34th Annual Meeting. New Orleans: Society of Gynecologic Oncologists/American College of Surgeons; 2003. Available at: http://www.sgo.org/meetings/abstract_search.cfm (accessed 1-15-04).

[31] Jayne CJ, Kaufman RH. Treatment of vulvar intraepithelial neoplasia 2/3 with imiquimod. J Reprod Med 2002;47:395–8.

[32] Cracchiolo BM, Heller DS, Wolff EC, Park MH, Clement PMJ, Hanauske-Abel HM. The hypusine form of eIF-5A in genital cancers: evidence for the pivotal function of translational control in malignancies. Abstr. # POS01. 35th Annual Meeting. San Diego: Society of Gynecologic Oncologists/American College of Surgeons; 2004. Available at: http://www.sgo.org/meetings (accessed 1-30-04).